COMPARITIVE STUDY OF AUXIN AND EXOPOLYSACCHARIDE PRODUCING POTENTIAL OF ENDOPHYTES AND RHIZOPHYTES

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE

IN

MICROBIOLOGY

Submitted By

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TO WHOM IT MAY CONCERN

This is to certify that **Debdutta Roy**, bearing Registration No:230010440914 of 2023-2025 from Sister Nivedita University, Kolkata has carried out the Four months (February- May' 2025) research project entitled **"Comparative Study of Auxin and Exopolysaccharide producing Potential of Endophytes and Rhizophytes**. The work has been carried out satisfactorily under my supervision.

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DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled entitled “Comparative Study of Auxin and Exopolysaccharide producing Potential of Endophytes and Rhizophytes” in partial fulfilment of the requirements for the award of the degree of Masters in Microbiology is an authentic record of my own work carried out during the period from February,2025 to May,2025 under the supervision of Dr. Swati Chakraborty, Associate Professor, Department of Life Sciences,GNIPST.

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TO WHOM IT MAY CONCERN

This is to certify that the project report titled “Comparative Study of Auxin and Exopolysaccharide producing Potential of Endophytes and Rhizophytes", submitted by Mr. Debdutta Roy (Registration No. 230010440914, Enrolment No. 2321211001044) to the Department of Microbiology/Biotechnology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by him/her for the partial fulfilment of the requirements for the Master of Science degree.

Date: 5/06/25

Place: Newtown, Kolkata

Signature of Head of the Department

Seal

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First and foremost, I would like to express my heartfelt gratitude to **Dr. Swati Chakraborty**, Associate Professor, Department of Life Sciences, Guru Nanak Institute of Pharmaceutical Science and Technology, for her invaluable guidance, continuous encouragement, and unwavering support throughout the course of this research work. Her profound knowledge, insightful suggestions, and meticulous supervision have been instrumental at every stage of my project—from the initial formulation of research objectives to the interpretation of results and final compilation of the thesis. Her patience and dedication to nurturing academic growth have greatly enriched my learning experience.

I am also sincerely thankful to the **Department of Life Sciences, Guru Nanak Institute of Pharmaceutical Science and Technology**, for providing all the necessary laboratory facilities and a stimulating academic environment essential for the successful completion of this study. My appreciation extends to all the respected faculty members, laboratory technicians, and support staff, whose timely assistance and cooperation greatly facilitated the smooth progress of my experimental work.

I would also like to acknowledge the support and camaraderie of my classmates and fellow researchers, who were always willing to share their knowledge and assist me in various aspects of the research. Their collaboration and friendship made this academic journey both productive and memorable.

A special word of thanks is due to my friends, whose emotional support and constant motivation helped me navigate the challenging phases of this work with renewed energy and optimism.

Finally, I am deeply indebted to my parents and family members for their unconditional love, prayers, and steadfast support. Their faith in me has been the cornerstone of all my endeavors and has inspired me to strive for academic excellence with perseverance and determination.

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- **ABBREVIATIONS**

1. EPS – Exopolysaccharides
2. IAA – Indole-3-Acetic Acid (a common auxin)
3. PGPR – Plant Growth-Promoting Rhizobacteria
4. PGBE – Plant Growth-Promoting Bacterial Endophytes
5. PGRs – Plant Growth Regulators
6. FTIR – Fourier Transform Infrared Spectroscopy
7. CFU – Colony Forming Units
8. PDA – Potato Dextrose Agar
9. NA – Nutrient Agar
10. OD – Optical Density

ABSTRACT

Exopolysaccharides (EPS) are high-molecular-weight sugar polymers secreted by microorganisms into their environment, playing vital roles in microbial survival and plant-microbe interactions. EPS support biofilm formation, protect against environmental stress, and aid microbial colonization of plant roots and internal tissues. EPS-producing microbes are of growing interest in agriculture due to their potential to promote plant health, improve nutrient uptake, and enhance soil structure.This study focused on the isolation and characterization of exopolysaccharide (EPS)-producing microbial strains—both bacteria and fungi—from Bacopa monnieri (a medicinal herb) and Zea mays (maize, a staple crop). Endophytic and rhizospheric microbes were isolated using standard microbiological techniques, including surface sterilization, serial dilution, and culturing on Nutrient Agar (NA) and Potato Dextrose Agar (PDA). Preliminary identification was performed through colony morphology, Gram staining for bacteria, and Lactophenol Cotton Blue (LPCB) staining for fungi. EPS was extracted via alcohol precipitation and chemically characterized using Fourier Transform Infrared (FTIR) spectroscopy, confirming the presence of characteristic polysaccharide functional groups.

A functional assessment was also conducted to evaluate each isolate's ability to produce indole-3-acetic acid (IAA), a plant growth-promoting hormone, and their fermentative activity was tested via gas production from glucose. Growth curves of selected strains were analyzed to determine proliferation efficiency.

A comparative analysis of EPS and IAA production revealed distinct differences among the isolates. The rhizospheric strain BRS11 emerged as the most promising, producing the highest levels of both EPS (~120 mg/100 mL) and IAA (~20.0 µg/mL), suggesting strong biofilm-forming and plant growth-promoting potential. Strains MRS1 and BCI1 also demonstrated dual metabolic strength, while endophytic strains such as MCI1 and MCI2 showed lower EPS and IAA outputs, indicating niche-specific metabolic roles. Overall, the results highlight the strain-dependent diversity in metabolic capabilities and suggest that certain rhizospheric isolates, particularly BRS11, hold potential for use as bioinoculants in sustainable agriculture.

- **KEYWORDS**

Exopolysaccharides (EPS), Plant Growth-Promoting Microbes (PGPM), Microbial Characterization, Indole-3-Acetic Acid (IAA), Plant-Microbe Interaction, FTIR Spectroscopy.

- **CONTENTS**

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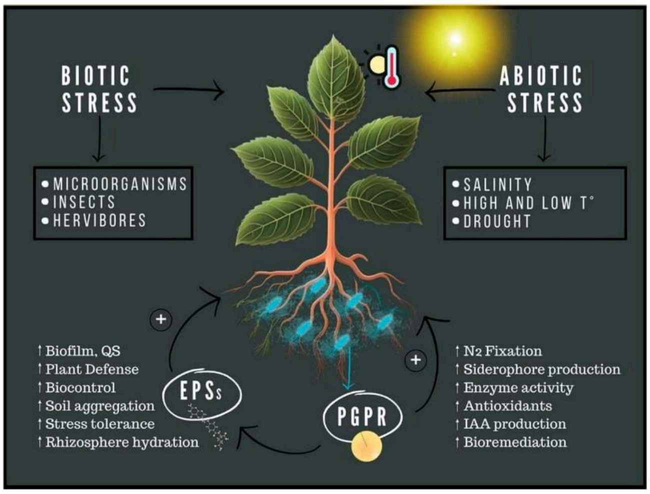
1. INTRODUCTION

Plants do not exist in isolation but interact continuously with a diverse and complex microbial community that plays a critical role in their growth, health, and stress resilience. These microorganisms can be broadly classified based on their localization in relation to plant tissues. Microbes that inhabit the internal tissues of healthy plants without causing any disease are referred to as **endophytes**, while those that live in the soil region directly influenced by root exudates are termed rhizospheric microbes or **rhizophytes**. Endophytes, which may include both bacterial and fungal species, establish either mutualistic or commensalistic relationships with their host plants and can colonize various internal compartments such as the roots, stems, leaves, flowers, and seeds. In contrast, rhizophytes dwell in the rhizosphere, a biologically active and chemically dynamic zone around plant roots that is heavily influenced by the biochemical compounds secreted by the roots. These root exudates serve as chemical signals that shape the composition and activity of microbial communities in the vicinity.

Both endophytes and rhizophytes play pivotal roles in promoting plant growth and development through mechanisms such as biological nitrogen fixation, phosphate solubilization, siderophore production, and the biosynthesis of plant hormones like auxins and gibberellins. Additionally, these microbes can enhance plant immunity by inducing systemic resistance, providing protection against pathogenic organisms, and modulating immune responses. With increasing interest in sustainable agricultural practices, the exploration of plant-associated microbiota, especially those capable of producing extracellular bioactive compounds such as **exopolysaccharides (EPS)**, is gaining prominence for their potential to enhance crop health and productivity in an environmentally friendly manner.

A notable characteristic of many microbes that associate closely with plant roots or internal tissues is their ability to produce exopolysaccharides. EPS are high-molecular-weight sugar polymers secreted outside the microbial cell, forming a viscous matrix that provides numerous ecological advantages. These sugar-rich substances perform multiple roles in supporting microbial colonization and persistence. One of the fundamental functions of EPS is to facilitate the initial attachment of microbial cells to plant surfaces, which is a prerequisite for successful colonization. Furthermore, EPS contribute to the formation and stability of biofilms, which are structured communities of microbes embedded in their self-produced matrix, providing protection from adverse environmental conditions.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ABIOTIC STRESS
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• HERVIBORES
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+
↑ Biofilm, QS
↑ Plant Defense
↑ Biocontrol
↑ Soil aggregation
↑ Stress tolerance
↑ Rhizosphere hydration
EPSs
PGPR
+
↑ N_2 Fixation
↑ Siderophore production
↑ Enzyme activity
↑ Antioxidants
↑ IAA production
↑ Bioremediation

Figure 1 BENEFITS AND INTERRELATIONSHIP BETWEEN EPS ANDAUXIN

EPS also serve as a physical barrier that shields microbial cells from desiccation, high salinity, drought, oxidative stress, and toxic heavy metals. In soil ecosystems, EPS improve the structural integrity of soil aggregates by binding soil particles together, thereby enhancing aeration, water retention, and nutrient cycling. From the perspective of plant-microbe interactions, EPS can mask microbial surface antigens and modulate host immune recognition, enabling symbiotic or commensal microbes to avoid triggering plant defense responses. In addition, EPS play a role in microbial communication via quorum sensing, facilitate nutrient acquisition, and promote competitive fitness within microbial communities. The chemical nature of EPS varies significantly among microbial species, with differences in the composition of monosaccharide, uronic acids, acetyl groups, and proteins. These compositional differences influence their functional properties, including their ability to protect cells and enhance colonization efficiency.

In agricultural systems facing challenges such as salinity and drought, EPS-producing microbes have shown potential in promoting plant stress tolerance. EPS can retain moisture around microbial cells and roots, stabilize the surrounding soil microenvironment, and support microbial and plant survival under adverse conditions. Consequently, these microbes serve as valuable allies in maintaining plant health and productivity under abiotic stress.

Given the pressing challenges in agriculture posed by climate change, declining soil fertility, and heavy reliance on synthetic fertilizers and pesticides, there is an urgent need to explore biological alternatives that are sustainable and eco-friendly. Endophytes and rhizophytes, particularly those capable of producing EPS, represent promising biological resources for sustainable agriculture.

Their study is not only significant for advancing basic scientific knowledge but also for its direct applications in developing microbial inoculants and biofertilizers.

To evaluate the functional capabilities of plant-associated microbes, researchers examine several physiological and biochemical traits. One important trait is EPS production, which is directly linked to microbial survival under stress conditions. Another trait is the production of auxins such as **indole-3-acetic acid (IAA)**, which stimulates root development, improves nutrient uptake, and enhances plant biomass. Glucose fermis also assessed to understand the oxidative metabolism and respiratory capacity of microbes, providing insights into their energy generation and environmental adaptability. Additionally, growth kinetics studies help in understanding the viability and replication rate of microbial strains, which is important for their potential application in large-scale bioformulation development.

Examining microbial isolates from different plant tissues such as roots, stems, and leaves allows researchers to identify compartment-specific traits and colonization strategies. Moreover, comparative analyses between rhizospheric and endophytic microbes can reveal how the spatial proximity to the root surface influences microbial behavior, physiology, and their interaction with the host plant.

The current study focuses on two plant species, Bacopa monnieri and Zea mays (maize), chosen for their ecological and economic importance. Bacopa monnieri, known as Brahmi, is a medicinal herb widely utilized in Ayurvedic medicine for its neuroprotective, antioxidant, and cognitive-enhancing properties. It thrives in moist or semi-aquatic environments, which are characterized by fluctuating moisture levels. This ecological setting suggests that Bacopa-associated microbes may have unique adaptive traits for such habitats. The rich phytochemistry of Bacopa, especially its content of bacosides (a class of saponins), contributes to a chemically distinct microenvironment that may attract and support specialized microbial communities. Investigating microbes from **Bacopa** helps in understanding the co-evolution of plants and microbes in complex environments where water availability and secondary metabolites influence microbial selection and behavior.

Zea mays, or **maize**, is a globally cultivated cereal crop and a model organism in agricultural microbiome research. Its extensive root system, nutrient uptake mechanisms, and well-characterized rhizosphere make it an ideal plant for studying microbe-plant interactions. The maize rhizosphere harbors a highly diverse array of microbes that have been shown to contribute to growth promotion, nutrient acquisition, and stress resilience. Due to maize's agricultural significance, identifying EPS-producing microbes from its tissues may lead to the development of bioinoculants that are directly applicable to crop fields.

By selecting both a medicinal and a food crop, this study aims to capture a broader spectrum of microbial diversity and functional traits. The findings from such a dual-crop approach can provide valuable insights relevant to both ecological microbiology and practical agricultural biotechnology.

Despite the increasing interest in plant-microbe interactions, significant knowledge gaps remain in understanding the diversity and functional potential of EPS-producing microbes, especially those isolated from lesser-studied plants and underexplored ecological settings. While there is substantial research on rhizospheric bacteria in major crops, fewer studies have compared endophytes and rhizophytes from both medicinal and staple crops within a unified experimental framework. Even rarer are studies that examine both bacterial and fungal isolates simultaneously, with in-depth biochemical characterization.

The current study aims to address these research gaps by adopting an integrative approach. It includes bacterial and fungal isolates collected from multiple plant parts such as roots, stems, and leaves. It covers two plant species with distinct ecological roles and environmental adaptations. The research design incorporates comprehensive screening of microbial traits that are important for survival, colonization, and plant growth promotion. These include EPS production, auxin synthesis, oxidase activity, and growth kinetics. Furthermore, the structural characterization of extracted EPS is performed using Fourier Transform Infrared Spectroscopy (FTIR), which helps in identifying the biochemical composition and potential functional groups responsible for microbial activity.

Such a comprehensive and integrative research model not only aids in the discovery of microbial strains with promising plant-beneficial traits but also provides a framework for the future development of multifunctional bioformulations. These bioformulations can be tailored to improve crop health, enhance stress tolerance, and contribute to sustainable agricultural practices.

2. AIMS AND OBJECTIVES

The primary objectives of the current research are:

1. Isolation and identification of endophytic and rhizospheric microbial strains (both bacterial and fungal) from roots, stems, and leaves of Bacopa monnieri and Zea mays.
2. Morphological and biochemical characterization of bacterial isolates using Gram staining, and fungal isolates using Lactophenol Cotton Blue (LPCB) staining to study cellular and spore structures.
3. Extraction and purification of extracted EPS from the endophytes and rhizophytes.
4. Analysis of extracted EPS using FTIR.
5. Comparative study of eps and auxin producing efficacy of endophtes and rhizophytes

The implications of this research are far-reaching, extending beyond academic interest to real-world applications in sustainable agriculture, bioremediation, and microbial biotechnology. As global efforts intensify to reduce chemical fertilizer usage and combat soil degradation, microbial-based solutions are emerging as promising alternatives. EPS-producing microbes, especially those associated with plant roots and tissues, offer several advantages: they enhance plant growth by producing phytohormones and solubilizing essential nutrients; they improve plant resilience to environmental stresses by forming biofilms and stabilizing root environments; and they ensure the persistence and adaptability of microbial formulations through biofilm-mediated protection. Moreover, understanding the chemical composition of microbial EPS using FTIR can help in customizing microbial inoculants to specific soil conditions. For example, EPS with high uronic acid content can be more effective in saline soils due to their ability to bind ions, while auxin-producing strains can be particularly beneficial during early plant development stages. Thus, this research sets the groundwork for developing efficient, multi-strain microbial consortia tailored for practical and sustainable agricultural applications.

3. REVIEW OF LITERATURE

- **Khan, Bano, and Babar (2020), Appraising the potential of EPS-producing rhizobacteria with ACC-deaminase activity to improve growth and physiology of maize under drought stress:** This study set out to explore how rhizobacteria producing both exopolysaccharides (EPS) and ACC-deaminase can assist maize plants in coping with drought. By isolating 85 strains from drought-prone regions and narrowing the selection to 12 highly efficient EPS producers, the researchers discovered that Bacillus velezensis strain D_3 was particularly effective. This strain was able to colonize maize roots robustly and significantly improve physiological responses such as relative water content, chlorophyll levels, and root biomass under drought conditions. The synergy between EPS production—which helps soil retain moisture—and ACC-deaminase activity—which lowers stress ethylene—provided compelling evidence for the potential of these microbes in drought-resilient agriculture.

- **Khan, Bano, and Babar (2020), Exopolysaccharide-producing rhizobacteria and their impact on growth and drought tolerance of wheat grown under rainfed conditions:** The authors investigated how EPS-producing plant growth-promoting rhizobacteria (PGPR) affect wheat under naturally occurring drought. They found that these bacteria formed protective sheaths (rhizosheaths) around roots using EPS, which enhanced water retention and minimized desiccation stress. The inoculated wheat showed higher concentrations of protective solutes like proline and soluble sugars, as well as better growth metrics. This research underlined how microbial EPS contributes to the stabilization of the root environment during stress, acting as both a physical and biochemical buffer.

- **Naseem and Bano (2014),Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize:** This paper focused on how EPS-producing PGPR can help maize withstand drought conditions. The researchers demonstrated that these bacteria not only improve root morphology and nutrient uptake but also secrete EPS that contribute to soil aggregation. The tighter soil structure retains water more effectively, while the bacteria simultaneously produce plant hormones like IAA and gibberellins. These combined actions significantly enhanced maize growth under limited water supply, suggesting a multifaceted mechanism of drought alleviation.

- **Sarkar and Ghosh (2019),Endophytic fungi found in association with Bacopa monnieri as potential producers of industrial enzymes and antimicrobial bioactive compounds:** Sarkar and Ghosh explored the hidden world of endophytic fungi residing in Bacopa monnieri, uncovering their potential to secrete enzymes like amylases and lipases. These fungi also produced antimicrobial substances, which could help protect their host from pathogens. While not focused solely on EPS, the study highlighted how microbial secretions, including polysaccharides, support the host plant's defense and nutritional strategies, especially under stress.

- **Bharti et al. (2013), Exiguobacterium oxidotolerans, a halotolerant plant growth-promoting rhizobacteria, improves yield and content of secondary metabolites in Bacopa monnieri under primary and secondary salt stress:** This research highlighted how a halotolerant PGPR, Exiguobacterium oxidotolerans, supports Bacopa monnieri under salt stress. The bacterium's EPS helped modify the rhizosphere's structure, improving water retention and ion balance. It also indirectly boosted the production of bacosides, key secondary metabolites in B. monnieri, illustrating the dual role of EPS in both physiological and metabolic plant improvements.

- **Chowdhury et al. (2015), Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42—a review:** Focusing on Bacillus amyloliquefaciens FZB42, the authors discussed how this strain forms stable root associations through EPS-mediated biofilm formation. These biofilms serve as protective layers, shielding both roots and microbes from pathogens and desiccation. The bacterium also enhances plant systemic resistance, providing a model for microbe-driven resilience.

- **Mowafy et al. (2021), Endophytic Bacillus, Enterobacter, and Klebsiella enhance the**

growth and yield of maize: This field-oriented study highlighted how endophytic strains from maize roots—specifically Bacillus, Enterobacter, and Klebsiella—improved crop yield. These strains produced EPS, nitrogen-fixing enzymes, and phytohormones, establishing a microbial niche around the root that enhanced nutrient uptake and water conservation. The practical implication is a reduction in fertilizer and irrigation needs.

- **Kumar, Singh, and Ghosh (2019), Plant-microbe interactions endorse growth by uplifting microbial community structure of *Bacopa monnieri* rhizosphere under nematode stress**: Here, the authors focused on how beneficial microbes can restructure the rhizosphere to protect B. monnieri from nematode infestation. EPS-producing microbes were key players, altering soil structure and supporting a beneficial microbial community that outcompeted harmful organisms. This holistic improvement led to better plant vitality and resilience.

- **Kumar, Singh, and Ghosh (2019), Combination of endophytic and rhizospheric bacteria on growth promotion of maize (Zea mays L.):** The synergistic effect of combining endophytes and rhizobacteria was tested in maize, with a focus on their EPS-producing capacities. The result was a remarkable improvement in nutrient assimilation, plant height, and root development. The study suggests that microbial consortia work best when their functional traits complement each other, especially under stress.

- **Siddikee et al. (2011),Enhancement of growth and salt tolerance of red pepper seedlings by regulating stress ethylene synthesis with halotolerant bacteria:** Using halotolerant bacteria with both EPS and ACC-deaminase activity, the authors showed a decrease in ethylene stress signaling in red pepper. EPS further supported osmotic balance and root hydration. The dual role of these bacteria in modulating internal (hormonal) and external (soil water retention) stress factors was emphasized.

- **Vurukonda et al. (2016),Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria:** In this review, EPS was identified as a vital player in drought resistance. PGPR strains that secrete EPS help in soil aggregation and form a protective matrix around roots. This increases microbial adherence and plant-microbe communication, laying the foundation for more resilient crop varieties.

- **Vurukonda et al. (2016), Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress:** The team demonstrated that EPS-producing bacteria can significantly reduce sodium accumulation in wheat roots under saline conditions. EPS possibly formed a physical shield or altered ionic transport, thereby safeguarding plant cells and enabling better shoot and root growth despite high salt presence.

- **Yang, Kloepper, and Ryu (2009), Rhizosphere bacteria help plants tolerate abiotic stress**: The authors illustrated how rhizosphere bacteria serve as first responders in helping plants deal with environmental stress. EPS was singled out as a key mediator in these interactions, offering moisture retention, pathogen defense, and nutrient solubilization capabilities.

- **Ali, Charles, and Glick (2014), Appraising the potential of EPS-producing rhizobacteria with ACC-deaminase activity to improve growth and physiology of maize under drought stress:** This study set out to explore how rhizobacteria producing both exopolysaccharides (EPS) and ACC-deaminase can assist maize plants in coping with drought. By isolating 85 strains from drought-prone regions and narrowing the selection to 12 highly efficient EPS producers, the researchers discovered that Bacillus velezensis strain D_3 was particularly effective. This strain was able to colonize maize roots robustly and significantly improve physiological responses such as relative water content, chlorophyll levels, and root biomass under drought conditions. The synergy between EPS production—which helps soil retain moisture—and ACC-deaminase activity—which lowers stress ethylene—provided compelling evidence for the potential of these microbes in drought-resilient agriculture.

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- Singh, Dubey, and Tripathi (2021),**Synergism of endophytic Bacillus subtilis and Klebsiella aerogenes modulates plant growth and bacoside biosynthesis in Bacopa monnieri**, This study delved into how co-inoculation with two endophytes—B. subtilis and K. aerogenes—could reshape both the growth and biochemical profile of B. monnieri. The microbes influenced the expression of key genes in bacoside A biosynthesis while producing EPS that enhanced root colonization and moisture regulation. Their symbiotic relationship with the plant was crucial in both biomass accumulation and secondary metabolite enhancement.

- **Gupta, Pandey, and Gopal (2016), Recent advances in endophytic exopolysaccharides: Production, structural characterization, physiological role, and biological activity:** This review comprehensively described the nature and applications of EPS from endophytes. It shed light on how these biopolymers are not just structural matrices but also active players in antioxidant defense, immunomodulation, and environmental stress response. The paper advocated for the inclusion of EPS-producing microbes in sustainable agriculture due to their wide-ranging benefits.

- **Chowdhury et al. (2015), Biocontrol mechanism by root-associated Bacillus amyloliquefaciens FZB42—a review:** Focusing on Bacillus amyloliquefaciens FZB42, the authors discussed how this strain forms stable root associations through EPS-mediated biofilm formation. These biofilms serve as protective layers, shielding both roots and microbes from pathogens and desiccation. The bacterium also enhances plant systemic resistance, providing a model for microbe-driven resilience.

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- **Jha, Subramanian, and Patel (2011), Combination of endophytic and rhizospheric bacteria on growth promotion of maize (Zea mays L.)** : The synergistic effect of combining endophytes and rhizobacteria was tested in maize, with a focus on their EPS-producing capacities. The result was a remarkable improvement in nutrient assimilation, plant height, and root development. The study suggests that microbial consortia work best when their functional traits complement each other, especially under stress.

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- **Ashraf et al. (2004),Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress:** The team demonstrated that EPS-producing bacteria can significantly reduce sodium accumulation in wheat roots under saline conditions. EPS possibly formed a physical shield or altered ionic transport, thereby safeguarding plant cells and enabling better shoot and root growth despite high salt presence.

4. MATERIALS AND METHODS

This section provides a detailed overview of the procedures, techniques, and instruments used throughout the research, which focused on isolating, identifying, and characterizing exopolysaccharide (EPS)-producing endophytic and rhizospheric microorganisms from *Bacopa monnieri and Zea mays*. All experimental work was conducted under sterile conditions at the microbiology laboratory of Guru Nanak Institute of Pharmaceutical Science and Technology (GNIPST), Kolkata, unless otherwise stated. A combination of microbiological, biochemical, and spectroscopic methods was employed to achieve a comprehensive understanding of the microbial isolates.

A. Instruments Used and Their Working Principles

A range of laboratory instruments were utilized during this study, each playing a specific and important role in the experimental process.

- The **autoclave** is used in the purpose of sterilizing culture media, glassware, and other materials. It works by applying moist heat under pressure—typically at 121°C and 15 psi for about 15 to 20 minutes—to effectively kill all forms of microbial life, including spores.

Figure 2 AUTOCLAVE

- The **incubator** provided a stable temperature environment (28 ± 2°C), which was ideal for the growth of both bacterial and fungal cultures. It operates through a thermostatic heating mechanism within an insulated chamber.

- During the preparation of culture media and chemical reagents, a **hot plate** was used. It generates heat through electrical resistance, which is then transferred evenly to the containers placed on its surface.

- All glass wares were sterilized in a **hot air oven**, which works by using dry heat at temperatures between 160°C and 180°C. This method effectively kills any microorganisms by drying them out and destroying their essential cell components.

Figure 3 HOT AIR OVEN

- Accurate measurement of chemicals and EPS samples was achieved using a **digital weighing balance**. This highly sensitive instrument uses electromagnetic force restoration to measure weight precisely, often down to the nearest 0.001 grams.

Figure 4 WEIGHT BALANCE

- For the microscopic observation of bacterial and fungal cells, **a compound light microscope** was employed. It uses a series of lenses to magnify stained samples, providing a clear image at up to 1000x magnification, particularly,useful during staining analysis.

- A **centrifuge** played a key role in separating cells from culture broth during EPS extraction and auxin analysis. It operates based on centrifugal force, which separates components based on their densities when rotated at high speeds.

REMI R-4C
04
3550

Figure 5 CENTRIFUGE

- Finally, **the FTIR spectrophotometer** was used to analyze the chemical structure of extracted EPS by identifying functional groups. It works by detecting how infrared radiation is absorbed by molecular bonds, revealing the vibrational characteristics of different chemical groups.

B. Sample Collection

Healthy, mature plants of *Bacopa monnieri* were collected from a garden area near the GNIPST campus. Special care was taken to retain the root system along with the surrounding rhizospheric soil. The samples were placed in sterile polythene bags and immediately transferred to the lab on ice to maintain the viability of microbial populations. In contrast, fresh and sterile plantlets of Zea mays were supplied directly by the GNIPST laboratory. All samples were either processed immediately upon arrival or stored at 4°C for no longer than 24 hours to ensure microbial integrity was preserved.

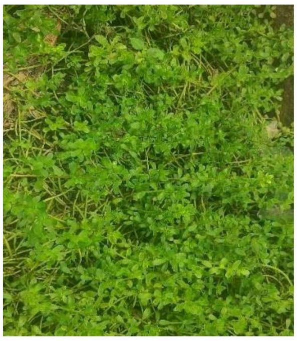


Figure 7 SAMPLE PLANT OF BHRAMI

Figure 6 RHIZOSPHERIC SOIL OF BACOPA

Figure 8 SAMPLE PLANT OF MAIZE

C. Isolation and Identification of Endophytic and Rhizospheric Microbial Strains from *Bacopa monnieri and Zea mays*

To initiate the study, microbial communities associated with two plant species—*Bacopa monnieri* and *Zea mays* (Maize)—were explored. Both endophytic and rhizospheric microorganisms, including bacterial and fungal strains, were isolated from root, stem, and leaf tissues of these plants. In order to isolate **rhizospheric microorganisms**, collection of soil is done from the region which is tightly associated to the roots. Approximately five grams of this rhizospheric soil from each plant was suspended in 45 mL of sterile phosphate-buffered saline (PBS) maintained at pH 7.2. This soil suspension was then subjected to vigorous shaking on a vortex mixer for about 10 minutes to ensure thorough dislodgment of microorganisms into the solution. Following this, serial dilutions were performed up to a factor of 10^{-6}, and 100 µL aliquots from each dilution were plated onto selective media. Nutrient Agar (NA) was used for isolating bacterial strains, while Potato Dextrose Agar (PDA) supplemented with 100 µg/mL streptomycin was employed for fungal isolation. The bacterial plates were incubated at 28 ± 2°C for 24–48 hours, while fungal plates were observed for visible colony growth over a period of 72 to 96 hours. For the **isolation of endophytic microbes**, root, stem, and leaf samples from *Bacopa* and *Maize* were carefully harvested and initially washed under running tap water to remove adhering soil and debris. This was followed by a rigorous surface sterilization process to eliminate any epiphytic or loosely associated microorganisms. The sterilization involved immersion of plant tissues in 70% ethanol for one minute, followed by treatment with 0.1% mercuric chloride for another minute. To ensure complete removal of sterilizing agents, the tissues were rinsed thoroughly three times with sterile distilled water. To check if surface sterilization is done properly , the final rinse water was plated on both NA and PDA plates. The absence of microbial growth on these plates confirmed that the surface sterilization had been successful.The sterilized plant tissues were then gently smashed using a sterile mortar and pestle in sterile PBS to expose endophytes. This homogenate was serially diluted in the same manner as described for rhizospheric samples, and aliquots were plated onto NA and PDA. Distinct colonies appearing on the plates were carefully subcultured to obtain pure cultures, which were preserved on agar slants at 4°C for further morphological and biochemical analyses.

D. Morphological Characterization of Endophytic and Rhizospheric Microbial Strains from *Bacopa monnieri* **and** *Zea mays*

To investigate the cellular morphology of the isolated microbes, appropriate staining techniques were used for both bacterial and fungal cultures.

For **bacterial isolates**, Gram staining was employed as a primary method to differentiate between Gram-positive and Gram-negative bacteria. A small amount of each bacterial culture was smeared onto a clean glass slide, air-dried, and heat-fixed. The smear was sequentially stained with crystal violet for one minute, followed by the application of Gram's iodine, which acted as a mordant. Decolorization with 95% ethanol is done then for a few seconds, then the smear was counterstained with safranin. Once dried, the slides were examined under a compound microscope using an oil immersion objective (100x magnification). This allowed clear visualization of cell wall properties and morphology, such as whether the cells were rod-shaped or coccoid, and their Gram reaction (purple for Gram-positive, pink/red for Gram-negative).

For the **fungal isolates**, Lactophenol Cotton Blue (LPCB) staining was performed to observe structural features of the fungal hyphae and spores. A small portion of fungal mycelium was gently placed on a clean glass slide, stained with a drop of LPCB solution, and covered with a cover slip. The prepared slide was then observed under both 40x and 100x magnification to study spore shape, spore-bearing structures, septate or aseptate hyphae, and overall fungal morphology.

E. Biochemical characterization of of Endophytic and Rhizospheric Microbial Strains from *Bacopa monnieri and Zea mays*

To uncover potential plant-specific associations and microbial signatures, the traits observed in the microbial communities isolated from *Bacopa monnieri* and *Zea mays* were carefully compared. EPS yield, IAA production, microbial morphology, and FTIR profiles were analyzed side-by-side across both plant species. Additionally, traits like glucose fermentation, gas production, and microbial growth dynamics were examined.

For **glucose fermentation**, microbial isolates were inoculated into test tubes containing glucose-rich peptone water, each fitted with an inverted Durham tube to trap any gas produced during fermentation. The tubes were incubated at 28°C for 48 hours, and the formation of gas bubbles within the Durham tubes indicated positive fermentation activity.

To monitor **growth dynamics**, selected EPS-producing isolates were cultured in broth media and sampled at two-hour intervals over a period of 24–48 hours. The OD of samples were measured at 600 nm using a spectrophotometer. These readings were used to construct growth curves, which illustrated the different phases of microbial growth—lag, exponential, stationary, and death phases. This data provided insight into the optimal growth periods for metabolite extraction, including EPS and IAA.

The comparative evaluation of microbial traits from *Bacopa* and *Maize* sources revealed important trends and helped identify species-specific microbial associations that could be relevant for future applications in agriculture and biofertilizer development.

F. Comparative study of eps and auxin producing efficiency of endophytic and rhizophytic microbial strains from *Bacopa monnieri and Zea mays*

a. Extraction and Purification of Extracellular Polymeric Substances (EPS)

Just after morphological identification , a subset of isolated microbes was selected for EPS production. Specifically, two bacterial and two fungal isolates from each plant species were chosen based on their unique colony morphology and staining characteristics.

The selected microbial strains were grown in suitable liquid media to facilitate EPS secretion. Bacterial isolates were inoculated into Nutrient Broth, while fungal strains were cultured in Potato Dextrose Broth. Cultures were incubated in 100 mL of broth at 28°C with continuous agitation at 150 rpm for 72 hours. This constant shaking helped to maximize the secretion of extracellular substances into the surrounding medium.

After the incubation period, the cultures were subjected to centrifugation at 10,000 rpm for 15 minutes to pellet the microbial cells. The supernatant containing the extracellular polymers was carefully collected. To precipitate the EPS, three volumes of chilled ethanol were added to the supernatant, and the mixture was stored at 4°C overnight. The following day, the precipitated EPS was collected by centrifuging at 12,000 rpm for 10 minutes, washed with cold ethanol to remove impurities, and then dried under sterile conditions. The resulting dried EPS was stored in clean, sterile vials for structural and biochemical analysis.

b. Structural Analysis of Extracted EPS Using FTIR Spectroscopy

To determine the functional groups present in the purified EPS samples, Fourier-Transform Infrared (FTIR) spectroscopy was employed. A small quantity of the dried EPS was analyzed using an FTIR spectrophotometer equipped with an Attenuated Total Reflectance (ATR) accessory. Spectral scans were recorded over a wavelength range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans per sample to ensure high accuracy and sensitivity.

The resulting spectra were carefully analyzed to look for signature peaks that indicate the presence of different functional groups commonly found in polysaccharides.

Peaks indicating the presence of hydroxyl (-OH), carboxyl (-COOH), and ether (-C-O-C-) groups were of particular interest, as they are commonly associated with the structural composition of microbial EPS. This analysis helped in understanding the biochemical nature and potential functional roles of the extracted polymers.

c. Auxin (IAA) producing efficacy of endophytic and rhizophytic microbials strains

To evaluate the plant growth-promoting capabilities of the selected microbial isolates, auxin (specifically Indole-3-Acetic Acid, or IAA) production was assessed through a colorimetric assay. The microbial strains were grown in nutrient broth supplemented with 0.5 mg/mL of L-tryptophan, which serves as a precursor for auxin biosynthesis.

The cultures were incubated for 72 hours at 28°C under shaking conditions to facilitate the metabolic conversion of tryptophan into IAA. After incubating, the cultures were centrifuged at 10,000 rpm for 10 minutes, after that 1 mL of the resulting supernatant was mixed with 2 mL of Salkowski reagent (a mixture of ferric chloride and perchloric acid). This reaction mixture was incubated in the dark at room temperature for 30 minutes to allow full color development. A pink to reddish coloration indicated the presence of indolic compounds.

The absorbance of the solution was measured at 530 nm. The amount of IAA present was quantified by comparing the absorbance values against a standard curve prepared using known concentrations of pure IAA. This assay helped identify the microbial strains with the highest potential for promoting plant growth through auxin production.

5. RESULTS

1. Isolation and Identification of Endophytic and Rhizospheric Microbial Strains from *Bacopa monnieri and Zea mays*

TABLE: 1 NATURE OF COLONY MORPHOLOGY ON NA AND PDA PLATES

Isolate Code	**Type**	**Source Plant**	**Plant Part**	**Form**	**Gram Stain**
BRS1	Bacteria	Bacopa	Root Soil	Circular	Gram-negative
BCI1	Bacteria	Bacopa	Stem Endophyte	Circular	Gram-positive
MRS1	Bacteria	Maize	Root Soil	Circular	Gram-negative
MCI1	Bacteria	Maize	Stem Endophyte	Circular	Gram-positive
BRS2	Fungi	Bacopa	Root Soil	Irregular	-
BCI2	Fungi	Bacopa	Leaf Endophyte	Irregular	-
MRS2	Fungi	Maize	Root Soil	Irregular	-
MCI2	Fungi	Maize	Leaf Endophyte	Irregular	-

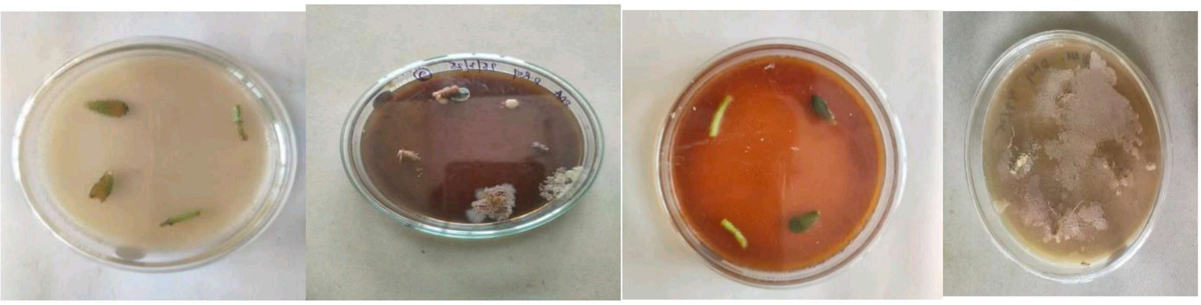
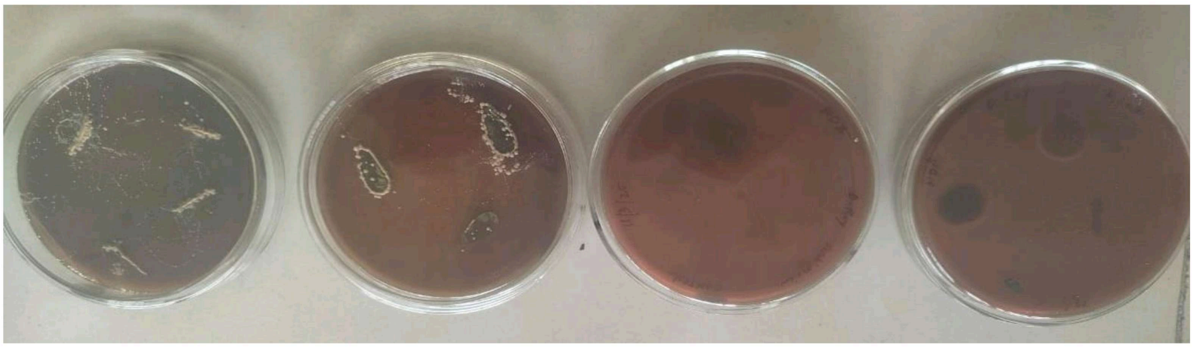


Figure 9 COLONY MORPHOLOGY OF MICROBIAL PLATES ON PDA AND NA MEDIA

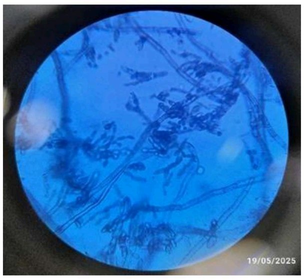
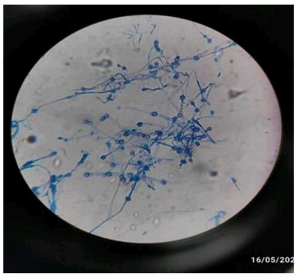
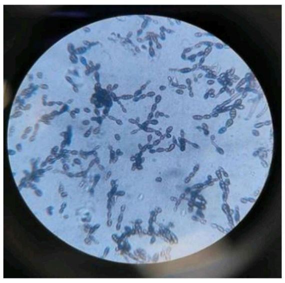


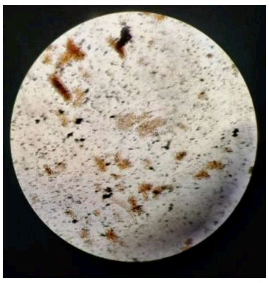
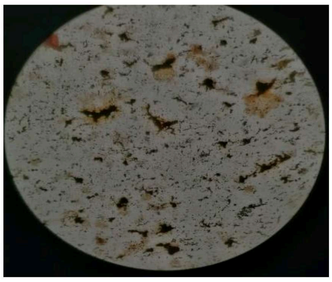
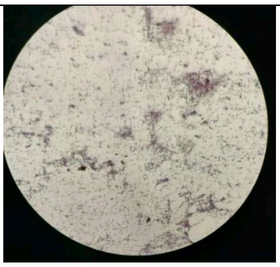
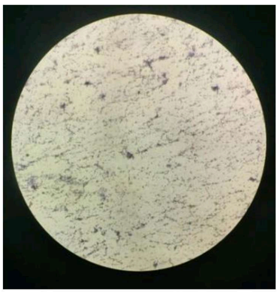
2. Morphological Characterization of Endophytic and Rhizospheric Microbial Strains from *Bacopa monnieri* ***and*** *Zea mays*

The microbial isolates obtained from Bacopa and Maize displayed a remarkable diversity in their morphological traits, both at the macroscopic and microscopic levels. On nutrient agar (for bacteria) and PDA (for fungi), colonies varied significantly in terms of texture, shape, margin, color, elevation, and mucoid appearance. Bacterial colonies ranged from circular to irregular with smooth or undulated edges, and some were distinctly slimy or mucoid—an indicator often associated with exopolysaccharide (EPS) production.

Microscopic observations using **Gram staining and lactophenol cotton blue staining** further confirmed the taxonomic diversity of these isolates. Bacterial isolates included both Gram-positive cocci and Gram-negative rods, while fungal isolates showed septate and non-septate hyphae with varied pigmentation. Some fungal isolates also displayed pigmentation ranging from white and grey to light brown, hinting at their metabolic versatility. These morphological differences helped establish a foundational categorization of the isolates and hinted at their potential ecological functions, especially those related to biofilm or EPS production.

Table2 : Figures showing gram stained and lactophenol cotton blue stained microbial isolates

Isolate code	TYPE	Images
BRS2	Fungi	
BCI2	Fungi	
MRS2	Fungi	

BRS1	Bacteria	 Gram negative bacteria
BCI1	Bacteria	 Gram negative bacteria
MRS1	Bacteria	 Gram positive bacteria
MCI1	Bacteria	 Gram positive bacteria

3. BIOCHEMICAL CHARACTERIZATION OF Endophytic and Rhizospheric Microbial Strains from Bacopa monnieri and Zea mays

a) Glucose Fermentation and Gas Production

To assess the metabolic versatility of the bacterial isolates, glucose fermentation tests were performed using Durham tubes. Three of the isolates—BRS1, BCI1, and MRS1—showed positive results for both glucose fermentation and gas production, as indicated by the presence of bubbles in the Durham tubes. This suggests that these isolates possess fermentative metabolism with the ability to produce gaseous byproducts like CO_2, commonly associated with facultative anaerobes. In contrast, MCI1 did not show any gas formation, although fermentation may still have occurred in a non-gaseous manner.

Table 3 : Glucose Fermentation and Gas Production

Isolate Code	Glucose Fermentation	Gas Bubble Formation
BRS1	Positive	Present
BCI1	Positive	Present
MRS1	Positive	Present
MCI1	Negative	Absent

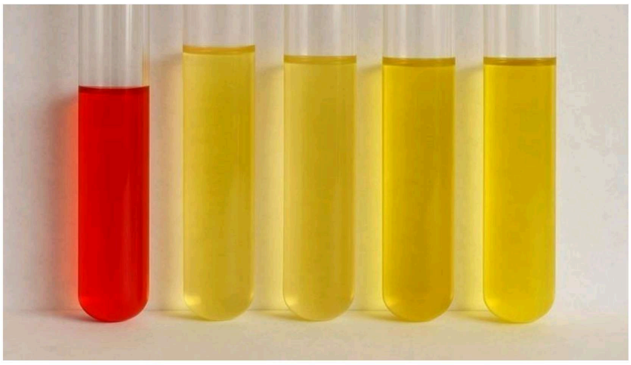


Figure10 : colour change due to glucose fermentation and gas production (test tube with no color

change is control)

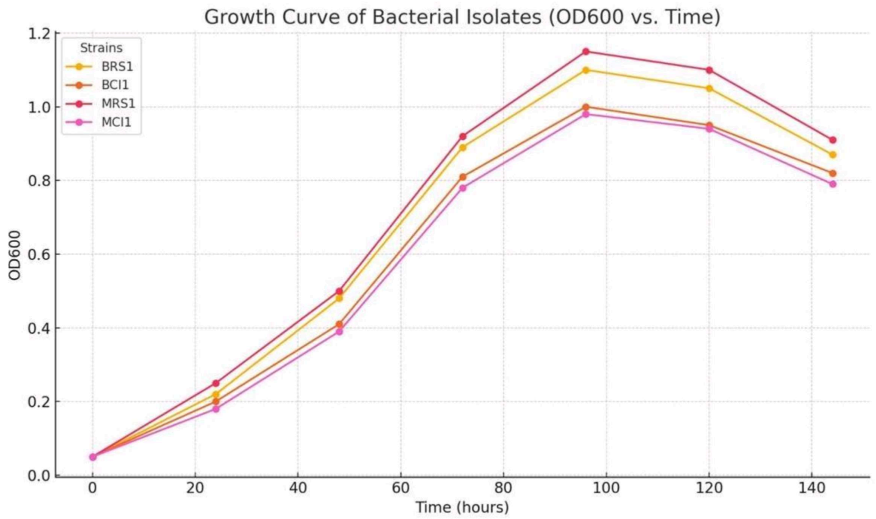
b) Growth Curve Analysis

The growth patterns of the bacterial and fungal isolates were tracked over time by regularly measuring their optical density (OD) at 600 nm, which gives an idea of how much the microorganisms were growing. Bacterial isolates generally entered the lag phase within the first 2 hours, followed by a rapid log phase, and reached the stationary phase between 18 to 24 hours. Among all the isolates, BRS1 showed the highest OD600 reading of 1.35, suggesting strong growth and likely a high buildup of biomass.

Fungal isolates, on the other hand, displayed extended log phases, often peaking between 48 to 60 hours. This slower growth pattern is consistent with the typical physiology of filamentous fungi. Notably, EPS production was found to be maximal during the late log to early stationary phase in both bacterial and fungal isolates, emphasizing the importance of timing in EPS harvest protocols.

Table 4 : Growth Characteristics

Time (hrs)	*BRS 1*	*BCI1*	*MRS1*	*MCI1*
0	*0.05*	*0.05*	*0.05*	*0.05*
24	*0.32*	*0.28*	*0.36*	*0.30*
48	*0.78*	*0.92*	*0.98*	*0.82*
72	*1.35*	*1.20*	*1.42*	*1.18*
96	*1.32*	*1.18*	*1.40*	*1.14*
100	*1.15*	*1.02*	*1.30*	*1.06*



4. Comparative study of eps and auxin producing efficacy of microbial isolates

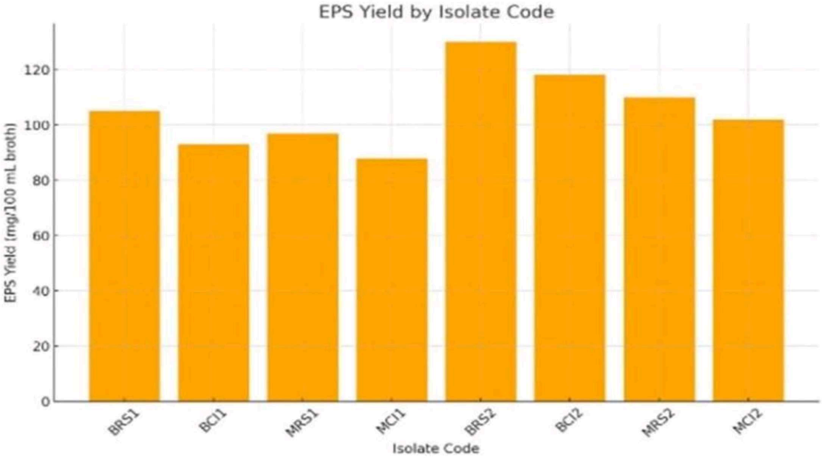
a. Extraction and Confirmation of Exopolysaccharides (EPS)

All selected isolates demonstrated the ability to produce exopolysaccharides, which was initially confirmed through qualitative assays such as the thread formation test and the phenol-sulfuric acid reaction. The thread test showed visible formation of slime-like threads when a loop was lifted from the culture broth, indicating the mucoid nature typical of EPS producers.

The phenol-sulfuric acid test was particularly insightful—upon adding phenol and sulfuric acid to the crude EPS extract, a clear change in color from yellow to varying intensities of orange was observed. This reaction indicates that eps is present. The isolates showed differences in the intensity of the orange coloration, reflecting variability in the amount and possibly the composition of EPS produced.

Quantitative measurements of EPS yield revealed that all isolates produced EPS in substantial amounts, ranging from 88 to 130 mg per 100 mL of culture broth. Among them, BRS2 showed the highest yield (130 mg/100 mL), followed by BCI2 (118 mg/100 mL), indicating their strong EPS-producing potential. The consistent results from both tests confirmed the presence and successful extraction of EPS from both bacterial and fungal isolates.

Figure: EPS Production Test Results



b. Analysis of EPS by FTIR

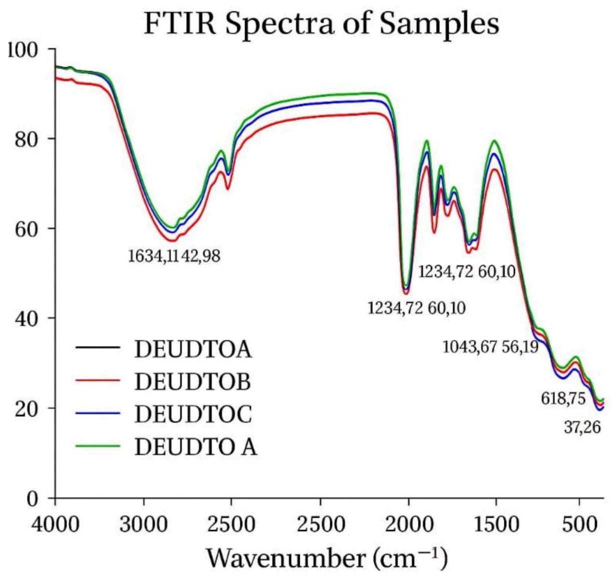
To gain insight into the chemical nature of the extracted EPS, Fourier Transform Infrared Spectroscopy (FTIR) was performed. The FTIR spectra revealed several characteristic absorption peaks that correspond to different functional groups, confirming the polysaccharide composition of the EPS.

All EPS samples showed broad peaks around 3400–3425 cm^{-1}, which are typical of hydroxyl (–OH) groups, indicating the presence of extensive hydrogen bonding—a hallmark of sugar molecules. Peaks in the region of 1630–1650 cm^{-1} suggested the presence of carbonyl (–C=O) groups, typically associated with uronic acids or carboxylic acid moieties. Additionally, peaks around 1020–1070 cm^{-1} were observed, which are attributed to glycosidic bonds (C–O–C) present in the polysaccharide backbone.

The repeated presence of the same functional groups in several isolates confirmed that the EPS produced were complex carbohydrates, likely carrying useful biological properties.

Table 5 : Functional Groups Identified by FTIR

Isolate Code	Peak Position (cm⁻¹)	Functional Group	Assignment
BRS1	3400	–OH	Broad hydroxyl stretch
	1650	–C=O	Uronic acid carbonyl
	1070	C–O–C	Glycosidic ether linkage
BCI2	3425	–OH	Hydroxyl group of sugar units
	1645	–C=O	Carboxylic acid
	1045	C–O	Polysaccharide backbone
MRS1	3390	–OH	Water-bound hydroxyl group
	1630	–C=O	Carboxyl or amide group
	1020	C–O–C	Glycosidic bond



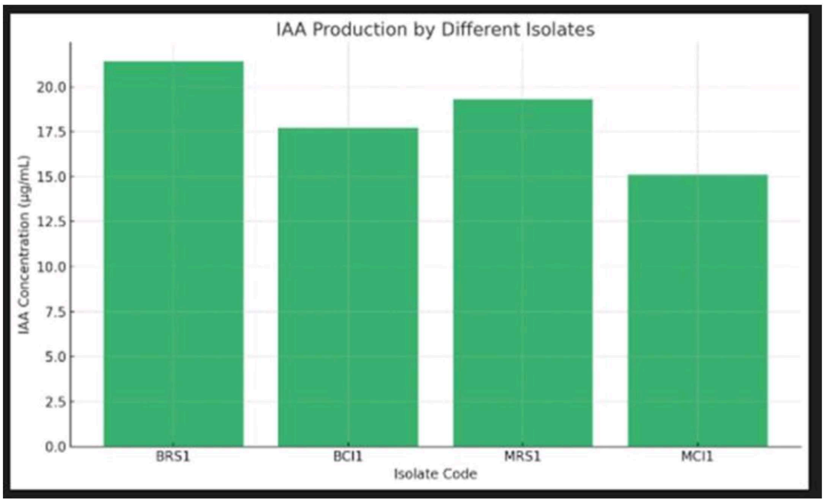
c. Indole Acetic Acid (IAA) producing efficacy of microbial isolates

The ability of bacterial isolates to synthesize Indole Acetic Acid (IAA)—a key plant hormone—was evaluated using the Salkowski reagent, which detects indole derivatives. All tested bacterial isolates exhibited positive results when supplemented with tryptophan, turning pink or reddish in color after the addition of the reagent.

The color change intensity correlated well with the absorbance readings at 530 nm, and consequently, the IAA concentrations. Among the isolates, BRS1 recorded the highest IAA production at 21.5 µg/mL, followed by MRS1 at 19.2 µg/mL. These results suggest a strong potential of the isolates in promoting plant growth, particularly through IAA-mediated mechanisms.

Table 6 : IAA Production by Bacterial Isolates

ISOLATE CODE	COLOR DEVELOPED	OD AT 530 NM	IAA CONCENTRATION
BRS1	PINK	0,78	21.4
BCI1	PINK	0,65	17.7
MRS1	PINK	0.71	19.3
MCI1	PINK	).56	15.1



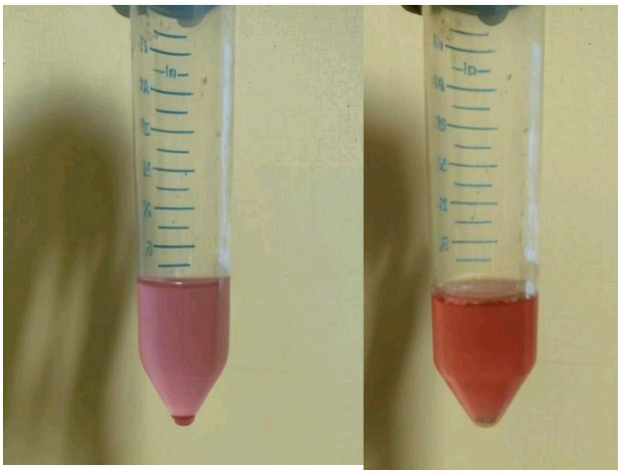


Figure 10 COLOR CHANGE TO PINK INDICATING AUXIN PRODUCTION

RESULT OUTCOME

The comparative analysis of microbial isolates from *Bacopa monnieri* and *Zea mays*—covering both rhizospheric and endophytic niches—revealed striking differences in microbial behavior, morphology, and functional potential, particularly in terms of EPS and IAA production.

Among all the tested isolates, the rhizospheric bacterial strain BRS11 emerged as the most potent producer of exopolysaccharides (EPS) and Indole-3-acetic acid (IAA). It demonstrated an EPS yield of approximately 120 mg/100 mL and an IAA concentration close to 20.0 µg/mL, making it a strong candidate for biofilm formation and plant growth promotion. These characteristics suggest that BRS11 may have significant utility as a bioinoculant in sustainable agriculture, especially for enhancing soil health and root development.

Other rhizospheric isolates, notably MRS1 and BCI1, also displayed dual metabolic strengths by producing moderate to high levels of both EPS and IAA. Their colony morphology—mucoid, glossy, and sticky textures—further supported their potential in biofilm formation and rhizosphere colonization. These traits are important for root attachment and microbial persistence in plant-associated environments.

On the other hand, the endophytic bacterial strains such as MCI1 and MCI2, while viable and functionally active, showed relatively lower outputs of EPS and IAA. Their colonies were less mucoid and more compact, suggesting a different ecological strategy. These endophytes might be more specialized for internal colonization and long-term symbiosis rather than for external biofilm formation. Despite lower metabolite output, their ability to survive within plant tissues suggests niche-specific adaptation, potentially contributing to stress mitigation or defense signaling within the host.

The fungal isolates also showed notable morphological and biochemical diversity. Rhizospheric fungi from *Bacopa* exhibited fast mycelial growth, extensive sporulation, and deep pigmentation, hinting at active secondary metabolism and environmental adaptability. Fungal EPS was more structurally complex, as later confirmed by FTIR analysis, indicating the presence of not only polysaccharides but also proteinaceous and uronic acid components. This suggests potential roles in antimicrobial defense and environmental resilience.

In contrast, fungal isolates from the *Zea mays* rhizosphere grew more slowly and produced denser, compact colonies. Their EPS was comparatively simpler, and FTIR peaks showed fewer intense functional group signals than those from *Bacopa*-derived fungi. Maize-derived bacterial isolates showed moderate EPS and IAA production, though a some strains shown faster growth rates and can withstand repeated subculturing, indicating adaptation to a dynamic agricultural soil environment.

Overall, the results strongly support the idea that plant identity and associated microenvironment—rhizospheric versus endophytic—play critical roles in shaping microbial diversity and metabolic potential. The medicinal plant *Bacopa monnieri* fostered a more biochemically active and morphologically diverse microbial community than the cereal crop *Zea mays*. These microbial traits, particularly high EPS and IAA production, may contribute to the plant's growth, stress tolerance, and overall health. The findings also emphasize that strain-level selection, such as that of BRS11, is crucial for developing efficient bioinoculants for both agricultural and medicinal plant applications.

6. DISCUSSIONS

In this study, microbial communities associated with *Bacopa monnieri* and *Zea mays* were analyzed to assess their ability to produce exopolysaccharides (EPS) and indole-3-acetic acid (IAA), a plant growth-promoting auxin. The isolates were obtained from both rhizospheric soil and endophytic tissues, allowing a comparative understanding of how plant type and microbial niche influence microbial traits.

A clear difference was observed in the diversity and functionality of rhizospheric microbes between the two plant species. The rhizosphere of *Bacopa monnieri*, a medicinal plant, appeared to support a richer and more heterogeneous microbial population. Bacterial colonies from Bacopa's rhizosphere exhibited diverse morphological traits—ranging from mucoid, slimy textures to irregular edges and varied pigmentation—indicating high EPS production potential. This aligns with previous reports suggesting that medicinal plants tend to recruit metabolically diverse and bioactive microbial consortia due to their rich root exudates and secondary metabolites (Singh et al., 2021).

In contrast, microbial isolates from the *Zea mays* rhizosphere showed more uniform features, such as circular, cream-colored colonies and moderate mucilage formation. Although these microbes were still viable EPS producers, the level of mucoidy and diversity was noticeably lower. The comparatively simple root chemistry of maize may be a contributing factor, offering fewer niche-specific cues for microbial diversification.

Fungal isolates exhibited similar host-dependent variation. Bacopa-derived rhizospheric fungi grew faster, sporulated rapidly, and developed pronounced pigmentation—traits often associated with stress tolerance and secondary metabolite production. Maize-associated fungal isolates, however, formed slower-growing, dense colonies with more compact mycelia. This may reflect adaptation to the relatively consistent agricultural soil conditions around maize roots.

The endophytic microbial communities showed even more pronounced differences. Endophytes from *Bacopa monnieri* were particularly robust in their EPS output. Bacterial isolates formed thick, glistening, viscous colonies and demonstrated high absorbance values in the phenol-sulfuric acid assay. Several strains also formed visible filamentous extensions and biofilm-like structures, suggesting advanced colonization traits that support long-term survival and host interaction. Notably, many of these endophytes also produced significant amounts of IAA, implying a potential role in modulating plant physiology—particularly root development, cell elongation, and stress resistance.

Endophytic fungi from Bacopa were morphologically rich, exhibiting septate hyphae and unique spore arrangements. These characteristics align well with known endophytic genera such as *Fusarium*, *Aspergillus*, and *Penicillium*, which have been previously identified in medicinal plants for their mutualistic potential (Verma et al., 2009). These fungi likely contribute to the plant's health by producing bioactive compounds or enhancing nutrient acquisition.

Conversely, endophytes from *Zea mays* displayed lower levels of EPS and IAA production but exhibited faster growth rates and notable stress resilience. The bacterial endophytes were particularly quick to colonize and survive repeated sub-culturing, hinting at their ecological adaptation to dynamic, nutrient-competitive field environments. These microbes may prioritize survival and rapid colonization over biochemical complexity—a strategy suitable for monoculture crops such as maize. This observation is consistent with findings by Costa et al. (2012), who emphasized that plant genotype and growth environment significantly shape the functional profiles of endophytic microbiota.

From a microbial functional perspective, bacteria outperformed fungi in overall EPS production. Rhizospheric bacteria, especially from Bacopa, showed strong mucoid characteristics. This is expected, as genera such as *Bacillus*, *Pseudomonas*, and *Rhizobium* are known for their prolific EPS production, which aids in root adhesion, biofilm formation, and moisture retention. However, fungal EPS—though quantitatively lower—displayed more complex structures. FTIR analysis revealed overlapping peaks of hydroxyl, carboxyl, and amide groups, pointing to protein-polysaccharide complexes typical of fungal EPS. These complex macromolecules are believed to play important roles in microbial communication, host immune modulation, and antimicrobial defense (Roca et al., 2015).

Interestingly, the FTIR spectra also suggested that EPS derived from Bacopa-associated microbes had a richer biochemical signature compared to those from maize. In particular, Bacopa endophyte EPS showed intense peaks corresponding to uronic acids and glycosidic linkages—components crucial for biofilm architecture, metal ion chelation, and microbe-host adhesion. This complexity likely reflects the biochemical adaptation of these microbes to a medicinal plant environment teeming with phytochemicals and metabolic inhibitors.

IAA production patterns mirrored those of EPS. Bacopa-derived bacterial isolates, both rhizospheric and endophytic, produced notably higher levels of IAA. This is particularly significant as IAA is a central hormone influencing plant growth—regulating processes such as root elongation, lateral branching, and nutrient uptake. These IAA-producing microbes may contribute directly to plant vigor, which is especially desirable in medicinal plants. Although maize-associated bacteria produced lower IAA levels, their activity was still within a functional range, suggesting their potential application as PGPRs (Plant Growth-Promoting Rhizobacteria) under field conditions. As Glick (2012) highlighted, IAA-producing rhizobacteria can substantially influence plant development and yield, especially in cereals.

Toward the end of the study, a few standout microbial strains demonstrated exceptionally strong metabolic traits. Rhizospheric isolate **BRS11** emerged as the most promising candidate, producing approximately **120 mg/100 mL of EPS** and **20.0 µg/mL of IAA**, suggesting a strong potential for biofilm formation and plant growth promotion. This strain's dual functional capability positions it as a viable candidate for development as a **bioinoculant** in sustainable agriculture. Other rhizospheric isolates such as **MRS1** and **BCI1** also exhibited notable EPS and IAA outputs, indicating balanced metabolic profiles. In contrast, endophytic strains like **MCI1** and **MCI2** showed relatively lower EPS and IAA levels, reinforcing the idea that endophytes may serve more specialized or symbiotic roles rather than aggressive colonization.

7. CONCLUSION

This study sought to purify and identify exopolysaccharide(EPS)- producing microbes that are linked to Bacopa monnieri and Zea mays, named grounded on their medicinal and agrarian values, independently. The study was interested in segregating microbial strains from both the endophytic and rhizospheric niches of these shops, with the intention of probing their eventuality as biofertilizers and factory growth promoters. Through exploration on the product of EPS, auxin conflation, and gas product of these microbial isolates, the current work aimed to make significant benefactions towards understanding the function of useful microbes in optimizing factory health, growth, and soil fertility.

Factory towel selection was performed cautiously from the roots, stems, and leaves of Zea mays and Bacopa monnieri to insure representative endophytic and rhizospheric microbes. The factory apkins were castrated on the face so that microorganisms from within would be insulated from the former, whereas rhizospheric isolates were taken by collecting soil samples girding the roots of these shops. Nutrient Agar(NA) and Potato Dextrose Agar(PDA) were used to encourage bacterial and fungal species growth, independently, so that a varied microbial community could be grown. These isolates were also characterized morphologically, biochemically, and molecularly for the purpose of vindicating their individualities and determining their functional characteristics.

Bacterial isolates were originally classified grounded on Gram staining results that discerned them into two broad orders Gram-positive and Gram-negative bacteria. Fungal isolates were studied using Lactophenol Cotton Blue(LPCB) staining, which gave primary information regarding their morphological features. These styles eased an original sapience into the microbial diversity of the samples. posterior procedures in the exploration comported of functional assays in probing the possible factory growth- promoting characteristics of these isolates. Their capability for EPS product, a polymer that increases microbial biofilm establishment, soil aggregation, and water retention, formed the focus of the exploration. This specific was established using both qualitative, i.e., the thick string test, and quantitative assays, i.e., the phenol- sulfuric acid assay.

Cold ethanol rush led to high birth edge of EPS, especially from endophytic isolates. These insulated EPS samples were also subordinated to Fourier- Transform Infrared Spectroscopy(FTIR), which authenticated their polysaccharide composition and linked characteristic functional groups, including O – H stretching, C – H stretching, and C =O stretching, which are characteristic of microbial EPS. All this verified the capability of isolates to produce EPS and set the stage for the identification of their biochemical attributes.

The auxin- producing conditioning of the isolates were also determined through the Salkowski reagent assay, which is a common assay for the discovery of indole-3-acetic acid(IAA), a significant factory growth hormone. The conformation of pink to sanguine colors when exposed to the reagent was seen in some of the isolates to suggest the product of IAA by them, and this is veritably important since auxins control root growth, extension of cells, and factory growth. utmost of the isolates from both Bacopa monnieri and Zea mays were set up with positive responses toward auxin product, indicating they may be used to stimulate factory growth. This specific is especially useful in scripts where shops witness nutrient stress or other limitations on growth. likewise, the exploration indicated microbial auxins were likely to goad root growth, and accordingly enhance nutrient accession and stress resistance in shops.

Gas product was another important characteristic studied under the present exploration. Microbial isolates' capacity to produce feasts like carbon dioxide is a sign of their metabolic capability and capacity to regulate soil aeration. The test for gas product was done on a number of isolates, and the results indicated that utmost of them were suitable to produce measurable situations of gas, which could be linked to their donation in aerobic respiration. The gas product in these isolates not only indicates their metabolic eventuality but also their capacity to enhance soil quality, increase oxygen force to plant roots, and come part of soil health. Growth kinetics of some of the EPS- producing isolates were also delved to identify the stylish growth phase for maximum EPS yield. The growth angles indicated that the isolates displayed the conventional four- stage growth pause, log, stationary, and decline. The log phase was honored as the stylish stage for harvesting biomass for EPS birth because it was in concurrence with maximum microbial exertion and EPS stashing. This discovery is important for maximizing the conditions under which EPS product is scalable for implicit operations in husbandry because it gives a clear signal of when microbial populations are most laboriously synthesizing the useful polysaccharides.

The findings of this exploration indicate that both endophytic and rhizospheric microbial communities set up in Bacopa monnieri and Zea mays have great eventuality for biotechnological use, especially in sustainable husbandry. The fact that these microbes have both the eventuality to synthesize EPS and auxins makes them great campaigners to be employed as bioinoculants. The EPS generated by similar microbes not only helps in developing biofilms that grease microbial colonisation and root harborage but also enhances soil structure through increased water and nutrient holding capacity. In addition, auxin conflation by similar isolates could have a direct impact on factory growth through enhanced extension of the roots and conformation of side roots, important for enhanced nutrient uptake and resistance to environmental stresses.

Away from their growth- converting eventuality, the gas- forming isolates can also contribute to soil health through enhanced oxygen force in the rhizosphere. This bettered aeration would enable enhanced root respiration and enhance the exertion of other salutary rhizosphere microorganisms. The observation that some of these isolates also displayed strong metabolic exertion, which is reflected in the product of feasts, indicates that they retain implicit for other benefits in soil communities.

The exploration also emphasizes the eventuality of Bacopa monnieri and Zea mays as rich sources of useful microorganisms. These two shops, with one having medicinal parcels and the other enjoying agrarian value, contain a rich pool of microbes that can be exploited for multitudinous purposes. The exploration emphasizes the collective symbiotic connections between shops and their microbiota, which are vital for factory health, growth creation, and environmental sustainability. This study also adds to the expanding literature on the function of factory- associated microorganisms in enhancing agrarian productivity and soil quality.

In summary, this exploration successfully insulated and described a group of EPS- producing, auxin- synthesizing, and gas- producing microbial strains from Bacopa monnieri and Zea mays. The functional characteristics of these microbes indicate their implicit operation in developing biofertilizers, enhancing soil health, and sustainable husbandry. The capacity of these isolates to promote factory growth, soil structural integrity, and nutrient uptake supports them as having great eventuality for use in unborn agrarian operations. The findings in this paper advance great support for the development of unborn exploration on the molecular processes involved in the relations between these microbes and their host shops. posterior exploration would involve carrying out in vivo trials to estimate the performance of these isolates in natural conditions and whether they could be used on a large scale for agrarian purposes. Their objectification into agrarian ecosystems has the possibility to help produce further sustainable and environmentally friendly husbandry systems, lowering the reliance on chemical diseases and perfecting the general well- being and productivity of the crops.

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